Keisuke KANAMORI*: **Studies on the sterility and size variation of spores in some species of Japanese *Dryopteris***

金森啓祐*: 日本産オシダ属胞子の変異と無配生殖について

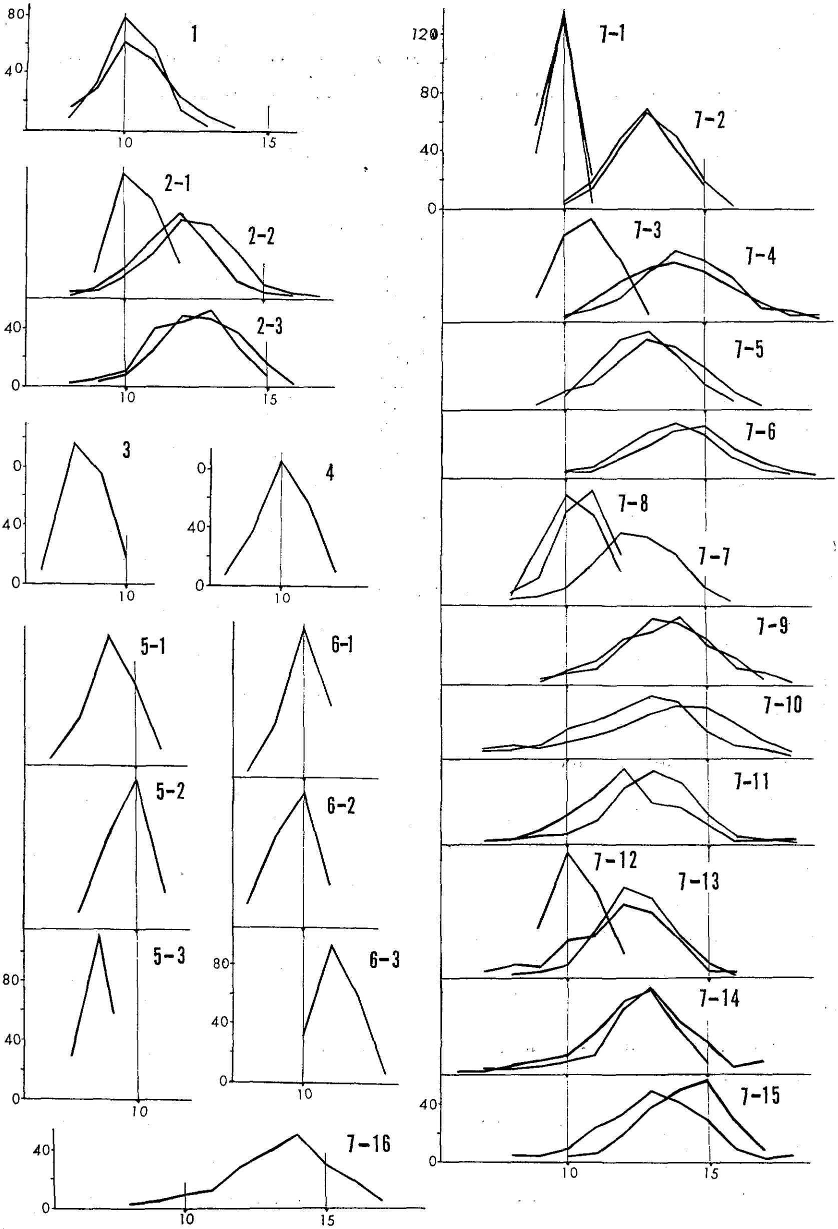
(Plates XIV–XVII)

Up to this time, there have been few reports on the variation of spore size, though the variation of spore shape was reported in the putative hybrids. Daigobo (1967) had compared and discussed the differences of spore size of the normal species with that of the apogamous ones and the putative hybrids described by Kurata (1964) in the genus *Polystichum* in Japan. Wagner and Chen (1965) described the variation of spore size and shape in the same sporangium and detected that they should be the putative hybrids in the genus *Dryopteris*. in U. S. A.. In this study, the author observed the variation of spore size of twenty-eight species belonging to *Dryopteris*. Additionally he calculated the ratio of aborive spores in each sporangium of the apogamous species in order to study how they were brought about. The author would like to express his sincere thanks to Prof. H. Ito for his valuable advice during this study.

Materials and Methods. The spores used for this observation were collected directly from the fronds in nature, not from the herbarium specimens, and they were fixed with Canada balsam. Except the evident abortive spores, two hundred viable spores were measured at random in each slide. The longest diameter of exospore was measured at the magnification of 400 times. Usual aceto-carmine squash technique was carried out for the observation of the abortive spores in a sporangium. Two hundred sporangia were observed in each sample.

Observation and Discussion. The variation curves of spore size show evident difference between those of the normal species and the apogamous ones. Seeing the Fig. 1, it is obvious that the graphs of the normal species

* Botanical Institute, Faculty of Science, Tokyo Univerity of Education, Tokyo. 東京教育大学理学部植物学教室.


1
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40
80
120
10
15

have steeper inclinations and narrower ranges than those of the apogamous ones. It is clear that the difference of the spore size between the maximum and the minimum one is less than 15.0μ in the normal species and is more than 22.5μ in the apogamous ones (Table 1). The range of variation in the apogamous species resembles those of the hybrids which shown by other investigators. Such differences are also reported between the normal species and the putative hybrids in *Dryopteris* by Wagner and Chen (1965) and in *Polystichum* by Daigobo (1967). They described about the characteristics of abortive spores of hybrids that the variation ranges of size were broad, with great numbers of uncommonly large and small spores in size; not typical "bean-shaped" but shperical, twisted or aggregative and so on in shape; and opaque and dark in color. Abortive spores of the apogamous species, however, did not indicate such characteristics in this study as well as those of *Polystichum* (Daigobo, 1967). They are typically "bean-shaped" spores and transparent. It was very convenient for observation of the exospores.

As shown in Table 1, the increment of spore size in this genus seems to have some connections with that of ploidal level of spore, especially in the couple of the related species. Such couples found in this study are as follows: Couple A: *D. tokyoensis* (1-pl.) 35.5μ and *D. atrata* (3-pl. apog.) 45.1μ; Couple B: *D. lacera* (1-pl.) 30.6μ and *D. uniformis* (2-pl.) 36.4μ; Couple C: *D. hayatae* (1-pl.) 36.3 μ and *D. sparsa* (2-pl.) 42.0μ; Couple D: *D. gymnophylla* (1-pl.) 36.8μ and *D. chinensis* (3-pl. apog.) 48.7μ; Couple E: *D. kinkiensis* (2-pl.) 38.6μ and *D. championii* (3-pl. apog.) 45.5μ. Considering from these couples, it might be said that the large difference of ploidal level of spore is parallel to the difference of spore size without exceptions in these cases. For instances, in the couples of normal species and the apogamous one, the difference of one ploidal level (Couple E) was about 7μ

Fig. 1. Variarion curves of spore size in *Dryopteris*. Vertical axis: Numbers of spores. Horizontal axis: Numbers of graduations of micrometer (1 division 3.75μ).
1. *D. sieboldii*. 2-1. *D. tokyoensis*. 2-2. *D. atrata*. 2-3. *D. commixta*. 3. *D. monticola*. 4. *D. austriaca*. 5-1. *D. crassirhizoma*. 5-2. *D. uniformis*. 5-3. *D. lacera*. 6-1. *D. sabaei*. 6-2. *D. hayatae*. 6-3. *D. sparsa*. 7-1. *D. gymnophylla*. 7-2. *D. chinensis*. 7-3. *D. saxifraga*. 7-4. *D. bissetiana*. 7-5. *D. pacifica*. 7-6. *D. sacrosancta*. 7-7. *D. varia*. 7-8. *D. sordidipes*. 7-9. *D. nipponensis*. 7-10. *D. erythrosora*. 7-11. *D. fuscipes*. 7-12. *D. kinkiensis*. 7-13. *D. championii*. 7-14. *D. hondoensis*. 7-15. *D. formosana*. 7-16. *D. gymnosora*.

Table 1. Size and ploidy of spore in *Dryopteris*.
Abbreviation a : apogamous

Name of species	Ploidy of species	Ploidy of spore	Size of spore	Difference (Max. & Min.)
Dryopteris sieboldii	2-*pl. a.*	2-*pl.*	38.8μ	27.5μ
tokyoensis	2-*pl.*	1-*pl.*	35.6	11.2
atrata	3-*pl. a.*	3-*pl.*	45.1	33.8
commixta			44.5	33.8
monticola	2-*pl.*	1-*pl.*	32.0	11.2
austriaca			38.0	15.0
crassirhizoma	2-*pl.*	1-*pl.*	33.8	15.0
uniformis	4-*pl.*	2-*pl.*	36.4	11.2
lacera	2-*pl.*	1-*pl.*	30.6	7.5
sabaei	2-*pl.*	1-*pl.*	37.5	11.2
hayatae	2-*pl.*	1-*pl.*	36.3	11.2
sparsa	4-*pl.*	2-*pl.*	42.0	11.2
gymnophylla	2-*pl.*	1-*pl.*	36.8	7.5
chinensis	3-*pl. a.*	3-*pl.*	48.7	22.5
saxifraga			40.5	15.0
bissetiana	3-*pl. a.*	3-*pl.*	47.6	30.0
pacifica			48.8	30.0
sacrosancta			53.5	30.0
varia	2-*pl. a.* 3-*pl. a.*	2-*pl.* 3-*pl.*	46.7	30.0
sordidipes	2-*pl.*	1-*pl.*	39.0	15.0
nipponensis	2-*pl. a.* 3-*pl. a.*	2-*pl.* 3-*pl.*	51.2	33.8
erythrosora	2-*pl. a.* 3-*pl. a.*	2-*pl.* 3-*pl.*	49.4	41.2
fuscipes	2-*pl. a.* 3-*pl. a.*	2-*pl.* 3-*pl.*	47.1	41.2
kinkiensis	4-*pl.*	2-*pl.*	38.6	11.2
championii	2-*pl. a.* 3-*pl. a.*	2-*pl.* 3-*pl.*	45.5	33.8
hondoensis	2-*pl. a.* 3-*pl. a.*	2-*pl.* 3-*pl.*	47.1	41.2
formosana	2-*pl. a.* 3-*pl. a.*	2-*pl.* 3-*pl.*	51.2	37.5
gymnosora	2-*pl. a.* 3-*pl. a.*	2-*pl.* 3-*pl.*	46.6	33.8

and that of two ploidal level (Couple A and D) was some 10μ. On the other hand, the difference of one ploidal level (Couple B and C) was almost 6μ in the couples of the normal species only. Mitui (1967) reported similar situation in the couple of *D. lacera* and *D. uniformis* (Couple B of this study). According to him *D. uniformis* has larger spores than *D. lacera*. He discussed the interrelation of those species by some characters of them and concluded that *D. uniformis* should be an autotetraploid derived from *D. lacera*. Moreover, he described some couples of putative autopolyploidal species from the morphological and cytological characters (Mitui, 1968). Couples C and D are contained within that description. From the situations of such couples it would be said that the other couples in this study may be autopolyploidal series.

When a young sporangium is squashed, thirty-two young spores are observed in it in the apogamous species. The author observed two hundred sporangia per an individual plant. Also he investigated the process and the causes of the appearance of the abortive or sterile spores. In this observation of young spores, three types of abortive or sterile spores were noticed. They are as follows: 1) Dwarf and shrunken type; This type of abortive spores were very tiny and shrunken. They had already shown wrinkled membranes on them while the nuclei are observed distinctly in the other viable large spores which are contained in the same sporangium (Pl. XIV-1). This type of abortion was encountered most frequently and most numerously in these three types in this study. This type also was reported in the normal species and the hybrids.

2) Irregularly dividing type; it seems that this type of abortion had brought from the last cell division which is not carried out under normal conditions during the sporogenesis. These conditions are not yet known. The spores formed in the sporangium in this case showed various sizes and shapes, even the trilete spores could be seen in an extreme case. This type was observed in some species in the group of *D. varia* and *D. erythrosora*. Pl. XIV-2 and Pl. XV-3 indicate the spores in one sporangium of *D. sacrosancta* and *D. hondoensis*, respectively. It is quite easy to receive some irregular divisions in these photos. Many fragments of spores show various degrees of sizes and shapes. Interesting one is the young spores of trilete-like form (See arrows in Pl. XIV-2). In Pl. XV-4, it could be counted 33 spores at the

first glance, but it should be 32 spores because the young two spores (trilete like), seen in the middle of this photo, were brought about from the redivision. Because the size of these smaller spores was almost half of the other larger ones, but the sum of them is almost equal size that of the rests. Pl. XVI-5, *D. sacrosancta*, also shows similar situation. Another figure of *D. hondoensis* (Pl. XVI-6) is a part of spores from a same sporangium. Three lumps of the tetrads show crumpled portions on the surface of each spore and their fragments.

Manton (1950) had described four types of spore formation in the apogamous species. In her third account on sporogenesis, she had described as follows: "The nuclei, after loss of spindle, may become irregularly lobed, cell walls partially crossing the cell may be laid down, often in relation to such lobes, and sometimes complete cleavages into two unequal portions containing different-sized pieces of the restitution nucleus may result.", and "Since the distribution of chromosomes to the constricted portions is certainly at random the nuclei so formed can hardly fail to be genetically unbalanced, and abortion of the resulting spores is therefore virtually certain." It is regretful that the author had no chance of observing of irregularly distributed chromosomes at meiosis in *Dryopteris*, though he found some such situations in the apogamous species of *Cyrtomium*, *Phegopteris* and *Athyrium*. The second type of abortion described in this study is similar to that of Manton's account on the unbalanced genetical condition. However, it is very different from the time when the Manton's case took place. It seems that the second type of abortion may be resulted from some unknown

Table 2. Abortion of spores

Species of *Dryopteris*	Numbers of abortive spores											
	1	2	3	4	5	6	8	10	12	14	16	20
D. atrata	7	17		28		12	20	3	5		3	4
D. chinensis	12	11		21			4		2			
D. pacifica	10	21	2	33	2	6	9		2			2
D. fuscipes	12	22		32		6	15	3	5		6	3
D. championii	13	23	2	15			7					3
D. hondoensis	14	17	5	28		7	12	6	10	2	9	4

conditions at the last two mitosis after the meiosis. It is well known that the cell divison is affected by sudden change of environmental factors of nature. However, in this case it seems that the abortion of spore is not always ascribed to the environmental changes, but genetical condition may be involved in the abortion of spores. Further studies are expected to research these factors on the abortion.

3) Imperfectly dividing type; this type of abortive or sterile spores appears the various conditions that the smaller spore is budding from the larger one, like the yeast (Pl. XVII-7, 8). In the other aggregations, spores of almost equal size are connected at various degrees, and show the shape like gourd (Pl. XVII-9). This type of abortive spores were observed in all species in this study. Not only one type, but two or all of three types are found frequently in same sporangium in many samples (Pl. XIV-1). It is known that the above mentioned three types are not the artifacts on the basis of the observation of similar three types in the matured spores which are collected from the field specimens.

The numbers of the abortive spores in a sporangium show an interesting result that the even numbers appear more frequently, especially the multiples of four, than the odd numbers in every species studied. The most frequent number observed was four in most species. It was observed in most case that all four spores from one spore mother cell became abortive (Pl. XIV-1). The occurrence of the odd number of abortive or sterile spores might be from the second or third type of abortion in many cases. However, the third type is more frequent than the second type. Though the appearance

in apogamous species.

per sporangium							abortive sporangia	abortive spores
22	24	25	26'	28	30	32		
4	5			3		9	60%	18.6%
				2		4	28	5.6
1	5		2	5	2	32	67	27.7
	3			4		12	61.5	18.0
	4		2	3	1	16	44.5	15.9
6	7	5		6	4	7	74.5	26.3

of sporangia which contained such abortive or sterile spores in two hundred samples showed high percent, it did not always show the accurate ratio of abortion and sterility because the various numbers of abortive or sterile spores were contained in each sporangium. In order to obtain the exact ratio of abortion and sterility, that of abortive or sterile spores to all the observed spores were calculated in every species (Table 2). These values are less than those of the sporangia in every species. The ratio of abortion and sterility seems to reveal the interrelation to the range of variation curve of spore size. For example, the abortion and sterility ratio of *D. chinensis* which has nar-

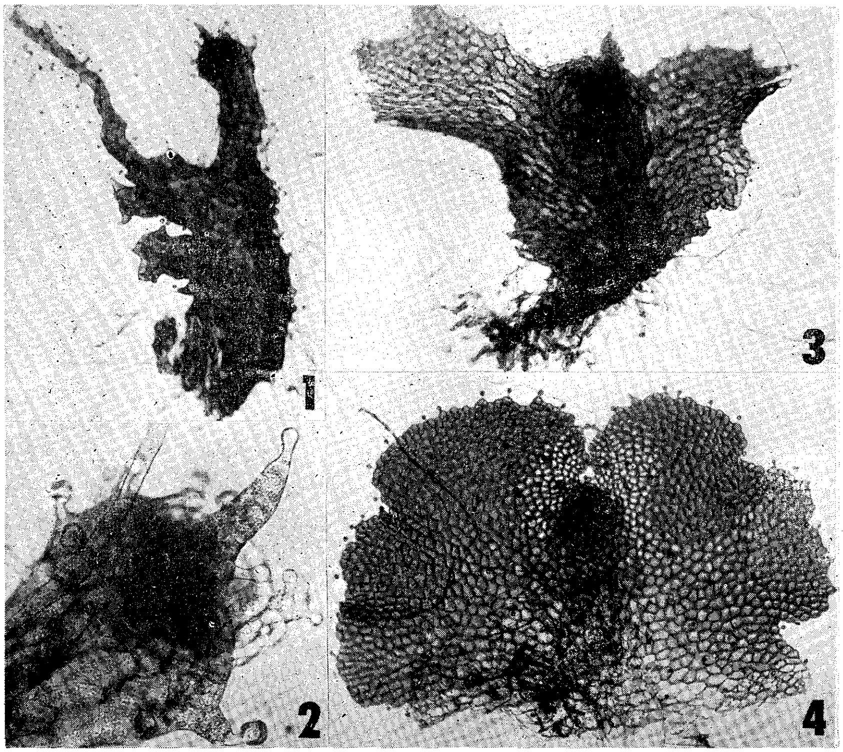

Fig. 2. Prothallium which bring about apogamous embryo.
1-3. *D. chinensis*. 4. *D. bissetiana*. 1. Irregular-shaped prothallium on which apogamous embryo is developing (See arrow.). ×16.5. 2. Magnified embryo of 1. ×165. 3. Heart-shaped prothallium on which apogamous embryo is developing. ×16.5. 4. Large heart-shaped prothallium. ×16.5.

rower range of variation (Fig. 1, 7–2) is lower than those of the other species of *D. varia-* and *D. erythrosora-* group which have broader ranges of variation. On the prothallia of some apogamous species, the occurrence of apogamous embryos were very different from species to species. Large numbers of apogamous embryos might be come out in a definite species, *D. chinensis* (Kanamori, 1967), and few numbers are found in the other species of *D. varia-* and *D. erythrosora*-group. In the former species apogamous embryos were brought about not only from large and heart-shaped prothallia but also from small and irregular-shaped ones (Fig. 2, 1–3). However, it is very interesting that in the latter ones the apogamous embryos developed only from the large and heart-shaped prothallia (Fig. 2–4). It would be said that the appearance of irregular-shaped ones in *D. chinensis* were ascribed not always to the genetical condition but to some physiological ones during the cultivation. On the other hand, it should be said that some of irregular-shaped prothallia of the other species were resulted from the genetical condition in many cases (Pl. XIV～XVII). Ito (1939) had described the species of *Dryopteris*, in Japan, Ryukyu and Formosa. He classified them into five Sections. His Sections and Subsections involved in this study as follows:

1. Sect. Eudryopteris H. Ito
   1) Subsect. Dichasium H. Ito (Fig. 1, 5–1～5–3)
   2) Subsect. Cycadinae H. Ito (Fig. 1, 2–1～2–3)
   3) Subsect. Monticolarum H. Ito (Fig. 1, 3)
   4) Subsect. Pycnopteris H. Ito (Fig. 1, 1)
2. Sect. Nephrocystis H. Ito (Figs 1, 6–1～6–3)
4. Sect. Polysticho-drys H. Ito
   1) Subsect. Erythro-variae H. Ito (Fig. 1, 7–1～7–14)
   2) Subsect. Formosanae H. Ito (Fig. 1, 7–15)
   3) Subsect. Gymnosorae H. Ito (Fig. 1, 7–16)
5. Sect. Lophodium C. Chr.
   1) Subsect. Austriacae H. Ito (Fig. 1, 4)

According to the system of his classification, each Section and Subsection shows two types of species constitution; namely only normal species in some groups and apogamous and normal species in the other groups. Adjusting the groups which contained only normal species to his system, next four groups should be considered like this, those are Subsect. Dichasium and

Monticolarum of Sect. Eudryopteris, Sect. Nephrocystis and Subsect. Austriacae of Sect. Lophodium. The variation curves of them show inclinations and smaller differences between the maximum and the minimum size. On the other hand, apogamous species are contained in Subsect. Cycadinae and Pycnopteris of Sect. Eudryopteris and Sect. Polysticho-drys, especially in Subsect. Erythro-variae. Almost of all of apogamous species in *Dryopteris* are included in his Subsect. Erythro-variae in Japan. The variation curves of them show gentle slopes and wider ranges. These remarkable differences are detectable in spore characters between normal species and apogamous ones as well as between the former and the hybrids. Therefore, from the viewpoint of spore morphology, Ito's system of *Dryopteris* seems a better one, but in this case there were some exceptions (*D. formosana* and *D. gymnosora*), so the author wishes to emphasize his grouping itself.

It could be said that the apogamy (obligate one) would be easily indicated roughly without cytological experiment from the morphological characters of young and matured spores. For example, it should be said that *D. pacifica* and *D. sacrosancta* might be triploidal apogamous species from the standpoint of spore morphology (variation curve, mean size and irregular division in sporogenesis).

References

Daigobo, S. 1967. Journ. Jap. Bot. **42**: 207–210. Hirabayashi, H. 1966. Journ. Jap. Bot. **41**: 11–13. —— 1967. Journ. Jap. Bot. **42**: 44–48. Ito, H. 1939. Polypodiaceae-Dryopteridoideae 1 in Nakai et Honda: Nova Flora Japonica no. 4. Kanamori, K. 1967. Journ. Jap. Bot. **42**: 111–118. Kurata, S. 1964. Science Report of the Yokosuka City Museum. No. 11: 17–41. Kurita, S. 1961. Bot. Mag. Tokyo **74**: 395–401. Manton, I. 1950. Problems of cytology and evolution in the Pteridophyta. Mitui, K. 1967. Journ. Jap. Bot. **42**: 28–32. —— 1968. The Science Report of the Tokyo Kyoiku Daigaku. Sec. B. **13**(203): 285–333. Wagner, W.H. Jr. and K.L. Chen 1965. Amer. Fern Jour. **55**: 9–29.

Explanation of Plates XIV～XVII

Figs. 1–9. Young spores from a sporangium. 1 and 7. *D. fuscipes*. 2 and 5. *D. sacrosancta*. 3, 4, 6 and 8. *D. hondoensis*. 9. *D. pacifica*.

1. Two types of abortive spores. Four dwarf spores and one irregularly divided spore are seen (See arrows). ×330.

2–6. Irregularly dividing abortive spores. 2. Various sizes and shapes are seen (See arrows). ×330. 3. Many fragments of spores are seen near the spores from which they are redivided. ×330. 4. Trilete like spores are seen (See arrows). In this photo, 33 spores from one sporangium are shown at a glance, but it seems that two smaller ones are produced by redivision of one spore. ×330. 5. Similar situation as 4. ×165. 6. Three tetrads which are forming smaller spores by irregular division (See arrows). ×330.

7–9. Imperfectly divided spores. ×660, respectively.

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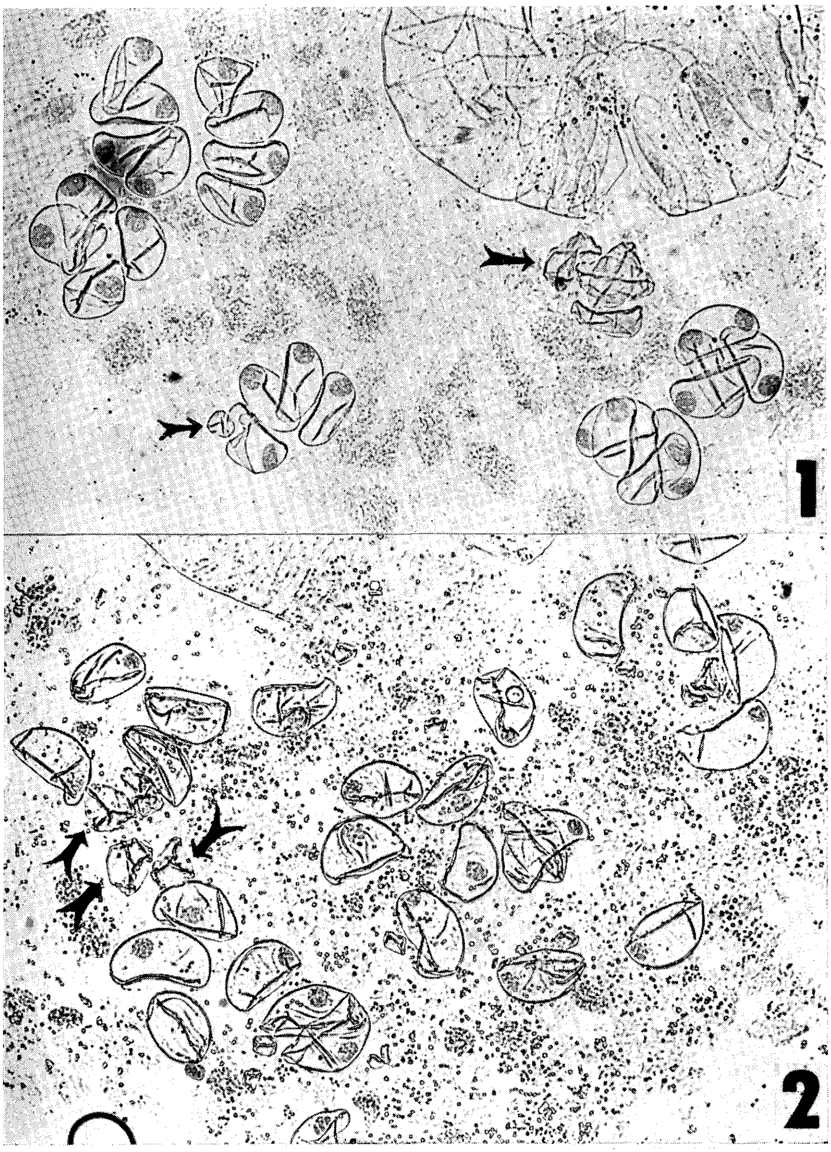
日本産のオシダ属の28種について胞子の大きさを測定し，その変異の幅は普通の種と無配生殖をする種とでは明らかに差があることが確められた。一胞子のう中に含まれる発育不全の胞子および不稔と思われる胞子を観察し，そのような胞子の生じる率と過程を考察した。その結果，これらの胞子の生じる率が高い種では変異の幅が広くなる傾向が見られた。また前葉体に無配生殖的な胚の発生する率と胞子の大きさの変異の幅とにはある関係が存するようである。すなわち，変異の幅の狭い種では胚の発生率が高く，変異の幅の広い種では発生率が低い。また胞子の大きさと胞子の倍数性との間にも相関関係が見られるようである。これらの胞子の諸性質から，細胞学的な観察なしに該種の生殖法が無配生殖であることがある程度推定できると考えられる。

○生薬貝母の調製法 (佐々木一郎) Ichiro SASAKI: A special technic to prepare the bulb of *Fritillaria verticillata* Willd. var. *thunbergii* Baker. to be used as drug.

生薬の貝母はバイモの鱗茎を乾燥したもので，日本では余り使用されないが，香港へ日本より輸出する重要な生薬の一つである。この鱗茎は普通の乾燥方法では仲々乾燥しないので，生薬には特殊の方法で乾燥調製して居り，主として奈良県で調製されて居る。畑より5月中旬～6月初旬に掘り取った貝母は大きさにより，大球（直径4 cm 以上）中球（直径 2 cm 以上）小球（直径 1.5 cm 以下）と大きさにより分別する。大球は薬用にしないで園芸店に売り，大きくてもせいぜい 3.5 cm 程度以下のものを生薬の製造に用い，来年の種球には 1～1.5 cm 程度のものを用いる。生薬としては小球の方が大球より上等品で高価なものが出来る。貝母を特殊の生薬剝皮機に入れて皮をむき，これに石灰乳をまぶし，特に芽の付いて居る球の内部に石灰乳が良く附着する様にする。これをむしろに重さならない様に並べて1～2日日陰に置き，良く石灰乳が浸み込んだら，これを乾燥機又は天日で乾燥して生薬にする。（津村研究所）



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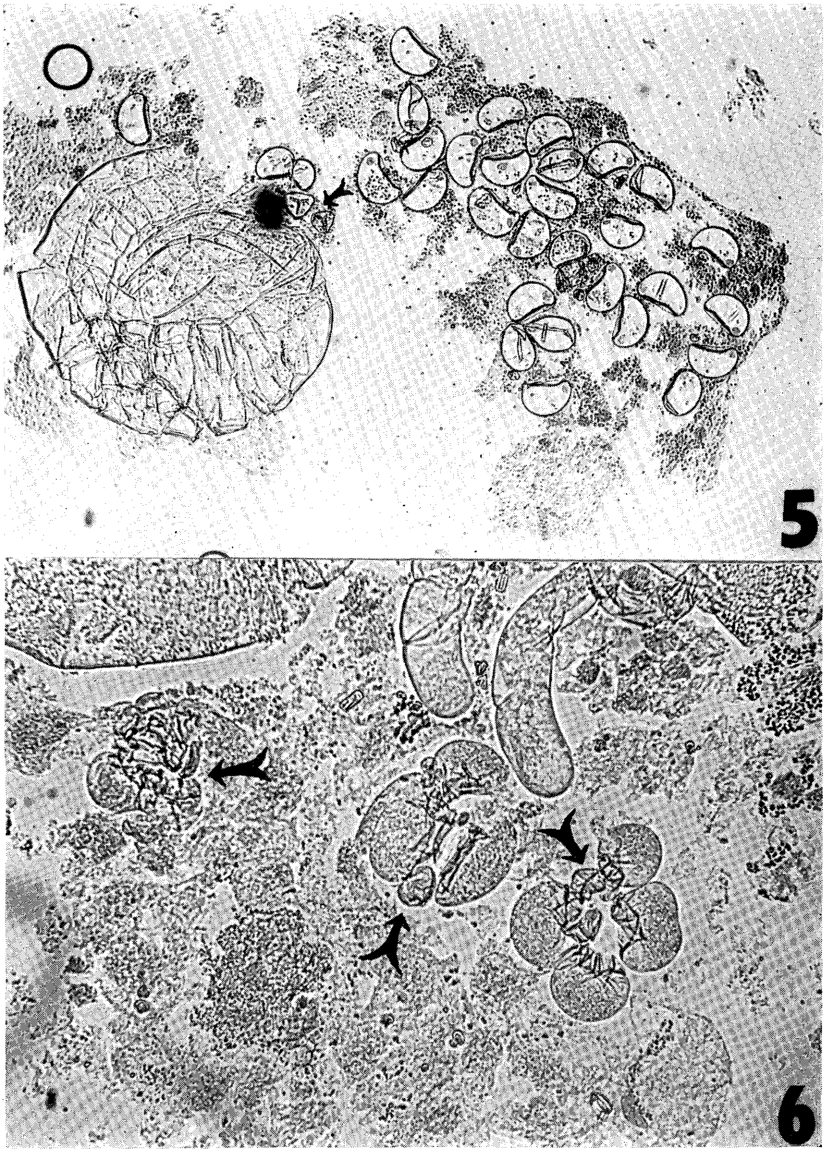

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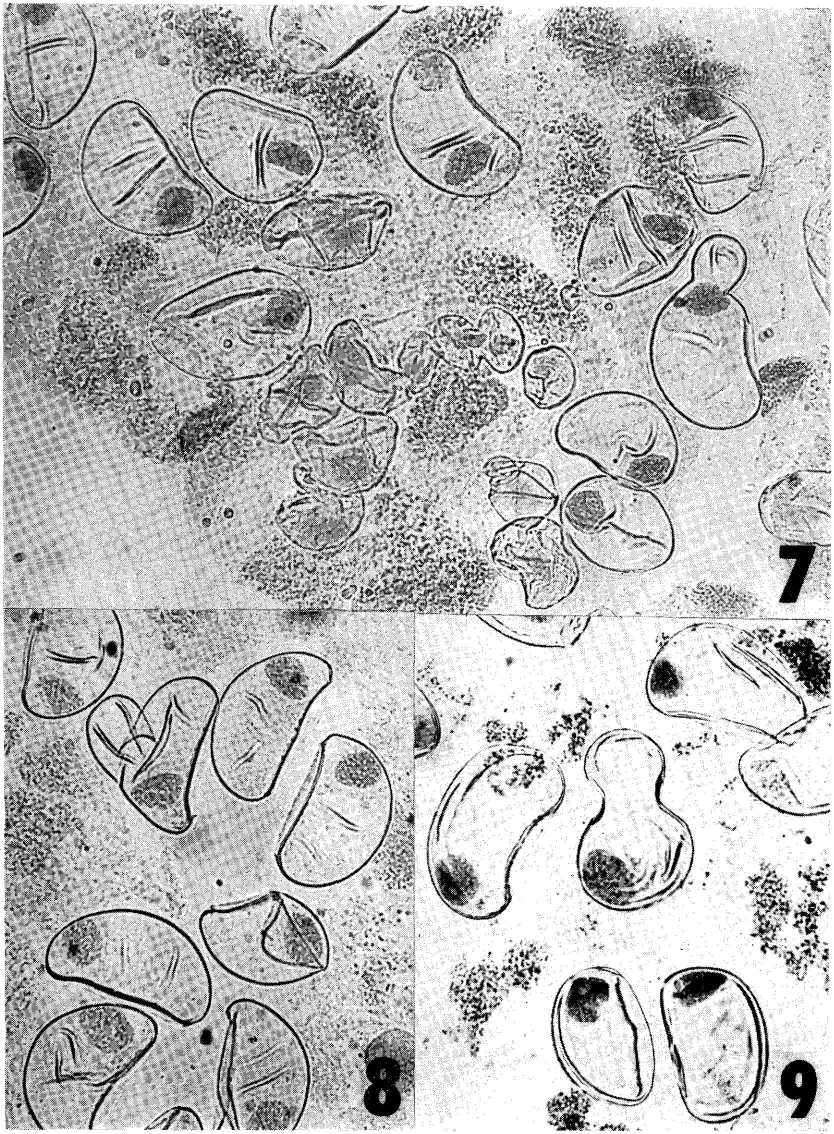

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